Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pesticide Biochemistry and Physiology 76 (2003) 37–45
PESTICIDE
Biochemistry & Physiology
www.elsevier.com/locate/ypest

Physiological basis for antagonism of clethodim by imazapic on goosegrass (*Eleusine indica* (L.) Gaertn.)

Ian C. Burke and John W. Wilcut*

Crop Science Department, Williams Hall 4402A, Box 7620, North Carolina State University, Raleigh, NC 27695-7620, USA

Received 24 April 2002; accepted 23 April 2003

Abstract

Greenhouse and laboratory experiments were conducted to determine the effect of imazapic on the herbicidal activity of clethodim on goosegrass. Imazapic did not affect absorption of [^{14}C]clethodim by goosegrass. Averaged across the two treatments of clethodim alone and clethodim plus imazapic, absorption was 36 and 89% of applied [^{14}C]clethodim at 0.5 and 96 h, respectively. The majority of [^{14}C]clethodim (79% of applied) was absorbed by 24 h. Translocation of ^{14}C was not affected by imazapic, and 3.6% of applied ^{14}C had translocated into the portion of the shoot below the treated leaf at 96 h after treatment. Metabolism of clethodim was not affected by the presence of imazapic. Three major metabolites of clethodim were detected in treated tissue at all harvest intervals. The majority (58%) of [^{14}C]clethodim was converted to a relative polar metabolite form 96 h after treatment, whether clethodim was applied alone or in the presence of imazapic. One day after treatment, the photosynthetic rate in plants treated with imazapic decreased below the rate in the non-treated check, and was less for 8 days, the duration of the study. These data suggest that the antagonism of clethodim by imazapic may be caused by imazapic reducing the photosynthetic rate of goosegrass and therefore the sensitivity of ACCase to clethodim.

Keywords: Absorption; Acetyl-coenzyme A carboxylase; Metabolism; Translocation

1. Introduction

Imazapic (AC 263,222) is an imidazolinone herbicide registered in peanut (*Arachis hypogaea* L.) for postemergence[1] (POST) control of broadleaf weeds such as cocklebur (*Xanthium strumarium* L.) and sicklepod [*Senna obtusifolia* (L.) Irwin and Barnaby] as well as purple (*Cyperus rotundus* L.) and yellow (*Cyperus esculentus* L.) nutsedge [1–3]. Plant death results from events occurring in response to acetolactate synthase [EC 4.1.3.18] (ALS) inhibition, which is a key enzyme in the biosynthesis of the branched-chain amino acids isoleucine, leucine, and valine [4].

Although imazapic also has activity on selected small annual and perennial grass species [5,6], it does not control goosegrass. Consequently, peanut

* Corresponding author. Fax: 1-919-515-5315.
E-mail address: john_wilcut@ncsu.edu (J.W. Wilcut).

[1] *Abbreviations used:* ACCase, acetyl-coenzyme A carboxylase [EC 6.4.1.2]; ALS, acetolactate synthase [EC 4.1.3.18]; POST, postemergence; LSS, liquid scintillation spectrometry; DAT, days after treatment.

0048-3575/03/$ - see front matter
doi:10.1016/S0048-3575(03)00062-2

growers do not rely on imazapic solely for grass control and typically use a selective postemergence graminicide to control grasses including goosegrass. Clethodim, an acetyl-coenzyme A carboxylase [EC 6.4.1.2] (ACCase) inhibitor, is a graminicide registered on cotton (*Gossypium hirsutum* L.), peanut, and soybean (*Glycine max* L.) [7]. As complexes of grass and broadleaf weeds are prevalent in agricultural fields, optimum application timings for herbicides having either grass or broadleaf herbicide activity can coincide. The effectiveness of imazapic on broadleaf weeds and sedges, and clethodim on annual and perennial grass weeds make the use of these herbicides applied postemergence in mixture a likely option for broad spectrum weed control in peanut. Furthermore, applying imazapic with clethodim would not only increase the spectrum of weed control but also reduce the cost associated with separate applications [8]. However, imazapic has been reported to cause antagonism of clethodim activity [9].

Several mechanisms have been proposed for antagonism of graminicides by other herbicides. Both absorption and translocation have been suggested as possible mechanisms for reduced grass control by graminicides when applied with broadleaf herbicides [10–15]. Further, it may also be possible that imazapic stimulates the activity of enzymes involved with herbicide metabolism, such as herbicide antidotes which induce glutathione-S-transferase enzymes in sorghum (*Sorghum bicolor* L.), and therefore increases the detoxification of clethodim [16]. Metabolism of the graminicide diclofop to a non-toxic metabolite confers selectivity to wheat (*Triticum aestivum* L.) [17].

Graminicides require actively growing meristematic regions for inhibition of ACCase [18]. ALS-inhibiting herbicides such as imazapic cause a wide variety of physiological responses in plants. One of the first responses to inhibition of ALS is a cessation of mitosis [19,20]. Inhibition of photosynthate transport is another symptom of ALS inhibiting herbicides in plants. Shortly after application of an ALS-inhibiting herbicide, neutral sugars accumulate in treated leaves because photosynthetic transport is inhibited [21]. However, ALS-inhibiting herbicides do not affect photosynthesis directly [4]. Therefore, this research was conducted to determine the basis of the antagonistic interaction between imazapic and clethodim on goosegrass [*Eleusine indica* (L.) Gaertn.], a common grass weed. The objectives of this research were to determine the effect of imazapic on the absorption, translocation, and metabolism of clethodim in goosegrass, and to examine goosegrass photosynthetic rate in response to an imazapic treatment.

2. Methods and materials

2.1. Plant material

Seeds of goosegrass were planted in a 1:1 mixture of pure sand and Norfolk loamy sand (fine-loamy, siliceous, thermic, Typic Paleudults) in 10-cm by 10-cm square plastic pots. Upon emergence, plants were thinned to one per pot. Plants were maintained in a glasshouse approximate daily minimum and maximum temperatures of 20 to 32 °C. A 14 h photoperiod of natural and supplemental metal halide lighting with an average midday photosynthetic photon flux density of 700 to 1400 $\mu mol\, m^{-2}\, s^{-1}$ was provided. All pots received 10 ml of a 25 $g\, L^{-1}$ commercial fertilizer (Peters Professional 20-20-20, Scotts-Sierra Horticultural Products, 14111 Scottslawn Rd., Marysville, OH 43041) at emergence and at 11 days after emergence.

2.2. Absorption and translocation

The study was conducted as a randomized complete block with a split-split-plot treatment design and four replications of treatments to evaluate absorption and translocation of clethodim alone and in the presence of imazapic. Main plots were harvest timings, sub-plots were plant portions, and sub-sub-plots were the two herbicide treatments of clethodim alone or clethodim plus imazapic. The study was repeated in time. At the 4-leaf growth stage, the leaf to which [^{14}C]clethodim was to be applied was covered with aluminum foil and formulated clethodim at 140 g ai ha^{-1} was applied to uncovered plant portions either alone or

in mixture with imazapic at 70 g ai ha^{-1}. Applications were made using a spray chamber equipped with a single 8001E flat fan nozzle (TeeJet Spray Nozzles, Spraying Systems, P.O. Box 7900, Wheaton, IL 60189) calibrated to deliver 160 L ha^{-1} at 200 kPa. Crop oil concentrate (Agri-Dex, 83% paraffin-base petroleum oil and 17% surfactant blend, Helena Chemical, Suite 500, 6075 Poplar Avenue, Memphis, TN 38137) at 1.0% (v/v) was included in both treatments. Immediately after application, five 1-μL droplets of [^{14}C]clethodim solution, containing approximately 1.7 kBq of radioactivity, were placed on the adaxial surface of the second fully expanded leaf of 4-leaf goosegrass. The two solutions contained either [^{14}C]clethodim (dissolved in acetonitrile) alone or with imazapic to correspond with the nonradiolabeled treatments. These solutions were prepared by diluting clethodim, labeled uniformly with ^{14}C in the phenyl ring [Ring-4,6-^{14}C] and a specific activity of 2.1 kBq μmol^{-1}, with either HPLC-grade water and formulated clethodim (Select), or HPLC-grade water, formulated clethodim, and imazapic at 0.1 μg ml^{-1}. Formulated clethodim was used to bring the total amount of clethodim applied to the treated leaf to 140 g ha^{-1}. Crop oil concentrate was included in both mixtures at 1% (v/v). The rates of clethodim and imazapic in the spotting solution were the same as in the solution applied using the spray chamber. Five μL of solution were added to liquid scintillation cocktail at the beginning of the ^{14}C-label application for each treatment. These samples were used to calculate the amount of ^{14}C applied to each plant as determined by liquid scintillation spectrometry (LSS) using a Packard TRI-CARB 2100TR Liquid Scintillation Spectrometer (Packard Instrument Company, 800 Research Parkway, Meriden, CT 06450).

Plants were removed from soil 0.5, 1, 2, 8, 24, 48, or 96 h after treatment (HAT) and were divided into treated leaf, roots, and aerial portions above and below the treated leaf. The treated leaves were rinsed for 20 s with 10 mL methanol:water (1:1, v/v) and 0.25% (v/v) nonionic surfactant (Induce nonionic low foam wetter/spreader adjuvant, 90% nonionic surfactant (alkylarylpolyoxyalkane ether and isopropanol), free fatty acids, and 10% water, Helena Chemical, Suite 500, 6075 Poplar Avenue, Memphis, TN 38137) to remove non-absorbed clethodim. A 1 ml aliquot of the rinse was added to 20 ml ScintiVerse SX18-4 Universal Liquid Scintillation Cocktail (Fisher Scientific, Fairlawn, NJ 07410) and radioactivity was quantified via LSS. All plant parts, including washed roots, were dried for 48 h at 40 °C, weighed, and combusted with a Model OX-500 Biological Material Oxidizer (R.J. Harvey Instrument, 123 Patterson Street, Hillsdale, NJ 07642). Radioactivity in the oxidized samples was quantified by LSS.

2.3. Metabolism

The metabolism study was conducted as a randomized complete block design with a split-split-plot treatment arrangement and four replications of treatments to evaluate metabolism of clethodim alone and in the presence of imazapic. Treatment design was the same as the absorption and translocation study. The study was repeated in time. Plants used for the metabolism experiments were grown, treated, and partitioned as described for the absorption and translocation experiments, with two exceptions. The amount of radioactivity applied to each leaf was 4.2 kBq, and the harvest intervals were 4, 8, 24, or 96 h. At harvest, plants were partitioned as previously described and were immediately placed in a freezer and stored at −30 °C until further analysis. Based on absorption and translocation experiments, only the treated leaf contained sufficient radioactivity for evaluation. Treated leaf sections were homogenized in 2–4 mL acetonitrile using Pyrex Tissue Homogenizer No. 7727-40 (Corning, Corning, NY 14831). The homogenate was then rinsed through a vacuum filtration apparatus with an additional 6–8 mL of acetonitrile. The residue and filter paper (Whatman #3 filter paper, Fisher Scientific, P.O. 4829, Norcross, GA 30091) were air dried, wrapped in aluminum foil to retain any dry matter recovered during the filtration process, and stored at room temperature. The homogenate was concentrated to 1.0 mL under a stream of air and stored at −30 °C until further analysis (Valent, personal communication). To evaluate the potential effects of the extraction process on herbicide

degradation, fresh plant leaves were harvested, spotted with 5 μL of the ^{14}C herbicide solutions and immediately processed in conjunction with the study samples. All herbicide extraction techniques were conducted on these freshly spotted leaves so that effects of the extraction process could be elucidated by later comparing pure [^{14}C]clethodim standard to the fresh-leaf extraction.

A 200 μL aliquot of each concentrated sample was fractionated by reversed-phase HPLC and quantified with in-line ^{14}C detection. To determine efficiency of both detection and extraction process using LSS, each injection, mobile phase solution, and Ultima-Flo M Flow Liquid Scintillation Cocktail (Packard Instrument, 800 Research Parkway, Meriden, CT 06450) was collected in its entirety and an aliquot taken, and the percent [^{14}C]clethodim and metabolites were determined by the ratio of each peak to the total ^{14}C of the injection.

The liquid chromatographic system consisted of a Model 715 Waters ULTRA WISP Sample Processor (Waters, 34 Maple St. Milford, MA 01757) equipped with a 200 μL sampling loop, two Model 6000 Waters Chromatography Pumps, and a Model 500 Radiomatic Flo-One Liquid Scintillation Spectrometer (Packard Instrument, 800 Research Parkway, Meriden, CT 06450) with a 100 μL flow cell. Gradients were controlled with a Model 680 Waters Automated Gradient Controller. An Allsphere ODS-1 5 μm 250 × 4.6 mm reversed phase column (Alltech Associates, 2051 Waukegan Rd., Deerfield, IL 60015) was used with a mobile phase gradient consisting of HPLC-grade water acidified with 1.0% acetic acid and HPLC-grade acetonitrile (Valent USA, personal communication).

2.4. Photosynthetic rate

To evaluate response of goosegrass photosynthetic rate to imazapic, a study was conducted as a randomized complete block with three replications of treatments. The study was repeated in time. At the 4-leaf growth stage, imazapic was applied at 70 g ha^{-1} using a spray chamber equipped with a single 8001E flat fan nozzle calibrated to deliver 160 L ha^{-1} at 200 kPa. Crop oil concentrate at 1.0% (v/v) was included in the spray solution.

Single leaf net photosynthetic rates were measured with a Model LI-6200 Portable Photosynthesis System (LI-COR, P.O. Box 4425, Lincoln, NE 68504). To ensure light saturation, photosynthetic rate was measured between 1100 and 1300 h immediately before treatment and 1, 2, 6, and 8 days after treatment (DAT) with imazapic. A 0.25-L chamber was used to enclose the middle portion of the second uppermost fully expanded leaf, and each measurement was made from the same leaf for the duration of the experiment. The gas exchange system was operated as a closed system to measure photosynthetic rate as a function of time to depletion of 3 ppm CO_2 [22]. The measurement was repeated three times per leaf each day. The area of leaf enclosed by the chamber was determined after measurement and used in the calculation of the photosynthetic rate.

2.5. Statistical analysis

Data were tested for homogeneity of variance prior to statistical analysis. Analysis of variance (ANOVA) was performed on absorption as a percent of applied ^{14}C over time. Linear, quadratic, and higher order polynomial equations were fit to the absorption data as percent of applied ^{14}C over time, by partitioning sums of squares [23]. Regression analysis was performed when significant absorption into the treated leaf of goosegrass was observed over time. Nonlinear models were used if ANOVA indicated that higher order polynomial effects of absorption were more significant than linear or quadratic estimates. Estimation used the Gauss–Newton algorithm, a nonlinear least squares technique [24].

For the translocation study, data were subjected to ANOVA with sums of squares partitioned to reflect a split-split-plot treatment structure and trial effects using the general linear models procedure in SAS [24]. The six harvest timings were considered main plots, the six plant portions were considered subplots, and the two spray mixtures (clethodim with or without imazapic) were considered sub-sub-plots. Statistical procedures for the metabolism study were similar

to those used in the translocation study. For photosynthetic rate measurements, data were subjected to ANOVA with sums of squares partitioned to reflect trial, day of measurement and treatment. In each experiment data were combined over trials. For all analyses, trial effects were considered random and mean squares were tested appropriately based on the treatment design [25]. Translocation, metabolism, and photosynthetic rate were separated by Fisher's Protected LSD test at $P = 0.05$.

3. Results and discussion

3.1. Absorption and translocation

Analysis of variance indicated that imazapic did not influence [^{14}C]clethodim absorption or translocation, thus data were pooled over the two herbicide treatments of clethodim and clethodim plus imazapic. Clethodim exhibited biphasic absorption, with 36% of the [^{14}C]clethodim absorbed in the first 0.5 h and absorption increased 79 percentage points over the following 23.5 h (Fig. 1).

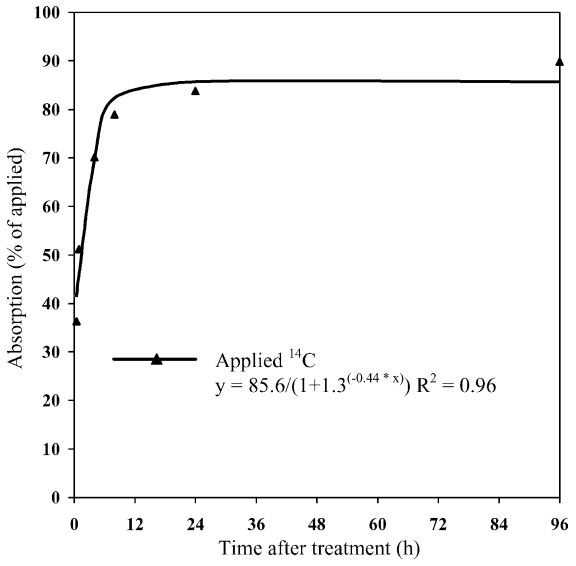

Fig. 1. Foliar absorption of [^{14}C]clethodim over time based on leaf wash recovery from goosegrass leaves averaged over herbicide treatments ([^{14}C]clethodim or [^{14}C]clethodim plus imazapic).

By 96 HAT, 89% of the [^{14}C]clethodim had been absorbed into the leaves of goosegrass. Clethodim and other cyclohexandione herbicides in general are rapidly absorbed, and have similar biphasic absorption patterns [12,26]. While absorption into the treated leaf increased over time, little ^{14}C translocated from the treated leaf to other plant portions (Table 1). By 96 HAT, 3.6% of applied ^{14}C had moved into the portion of the shoot below the treated leaf, the location of the intercalary meristem and the site of action of ACCase inhibitors [18,27,28], while 4.7% of applied ^{14}C had moved to the shoot above the treated leaf. Other researchers have also reported that cyclohexanedione herbicides are readily absorbed into leaf tissue with only limited translocation out of the treated leaf [12,29].

Although our data suggest that imazapic does not affect translocation of clethodim out of the treated leaf, others have noted differences in translocation of graminicides when mixed with an ALS-inhibiting herbicide [10,13,30]. It has been suggested that ALS-inhibiting herbicides affect photosynthate transport processes [21], and may therefore affect movement of the graminicides or the corresponding bio-activated metabolite to the site of action (intercalary meristem). It should be noted that the amount of cyclohexanedione herbicide required for ACCase inhibition is very low, with a calculated I_{50} value for sethoxydim of 2.9 μmol and greater than 90% inhibition at 100 μmol [28,31,32]. Therefore, the small differences in translocation reported in other studies may not account for the magnitude of herbicide antagonism resulting in lack of control. A mechanism of antagonism different from, or in addition to, translocation may account for the reduction in grass control observed in efficacy studies with clethodim and imazapic [9].

3.2. Metabolism

Analysis of variance indicated that imazapic did not influence [^{14}C]clethodim metabolism, thus data were pooled over the two herbicide treatments of clethodim and clethodim plus imazapic. Three major metabolites of clethodim were detected in treated tissue at all harvest intervals, while no

Table 1
Influence of harvest timings of 0.5, 1, 4, 8, 24, and 96 h after treatment on the distribution of absorbed ^{14}C based on oxidation of treated goosegrass averaged over herbicide treatments of [^{14}C]clethodim and [^{14}C]clethodim plus imazapic

Harvest timing (h)	Applied ^{14}C[a]			
	Treated leaf (%)	Shoot above (%)	Shoot below (%)	Root (%)
0.5	36	0.1	0.1	0.1
1.0	50	1.2	0.3	0.2
4.0	70	1.6	2.3	0.4
8.0	79	1.6	2.4	0.4
24	84	1.8	3.2	0.5
96	89	4.7	3.6	0.6
LSD (0.05)	7	1.6	1.1	0.2

[a] Clethodim was applied at 140 g ha^{-1} alone or in mixture with imazapic at 70 g ha^{-1}.

Table 2
Influence of harvest timings of 4, 8, 24, and 96 h after treatment on the proportion of absorbed ^{14}C-label metabolites in treated leaves of goosegrass averaged over herbicide treatments of [^{14}C]clethodim alone and [^{14}C]clethodim plus imazapic

Time (h)	Distribution of absorbed ^{14}C in the treated leaf[a]		
	Metabolite (retention time in min)		
	A (4.0)	B (14.0)	C (26.0)
	% of total detected metabolites		
4	14	42	36
8	26	46	19
24	32	42	14
96	58	23	3
LSD (0.05)	7	6	5

[a] Clethodim was applied at 140 g ha^{-1} alone or in mixture with imazapic at 70 g ha^{-1}.

[^{14}C]clethodim (retention time of 35.5 min) was recovered at any harvest interval (data not shown). Of the three metabolites, the greatest percentage of total metabolite at the 4 h harvest consisted of metabolite 'C' (retention time of 27 min) (Table 2). From the 4 h to the 96 h harvest, metabolite 'C' decreased from 36 to 3% of total detected metabolite. Metabolite 'B' (retention time of 14 min) also decreased as percentage of total metabolite from the 48 h harvest to the 96 h harvest. Metabolite 'A' (retention time 4.0 min) increased from 14% of total metabolite at the 4 h harvest to 58% of total metabolite at the 96 h harvest.

No metabolites of clethodim have been previously described, however, the metabolites of a structurally related compound, allyoxidim, have been elucidated [33,34]. Clethodim could be transformed similarly in plant tissue. The sulfur in clethodim is available for oxidation to the corresponding sulfoxide and sulfone. Sulfur is readily oxidized in other pesticidal molecules [35], and metabolites 'B' and 'C' may correspond to the sulfone and sulfoxide, respectively, of clethodim. In this study, the metabolism of clethodim proceeded rapidly, as has been reported for sethoxydim as well. Within 24 h, 98% of sethoxydim was degraded in tolerant as well as in sensitive species [29,36]. Metabolite 'A' is relatively polar compared to the other two metabolites and clethodim as determined by its retention time. After oxidation, herbicide metabolites are typically conjugated to a more polar product in preparation for sequestration [18]. Both major families of graminicides, the cyclohexanedione and aryloxyphenoxypropionate herbicides, are metabolized at similar rates alone or when applied in the presence of other herbicides including ALS-inhibiting herbicides [11–13,26].

3.3. Photosynthetic rate

Immediately before an application of imazapic, rates of photosynthesis were similar for all goosegrass plants (Fig. 2). One DAT, the photosynthetic rate in plants treated with imazapic had decreased by 5.9 μmol CO_2 m^{-2} s^{-1}, and was less at 2, 6, and 8 DAT. The photosynthetic rate in non-treated goosegrass continued to increase. Thus imazapic appears to reduce photosynthetic rate of goosegrass. The reduction in photosynthetic rate of imazapic-treated goosegrass compared with

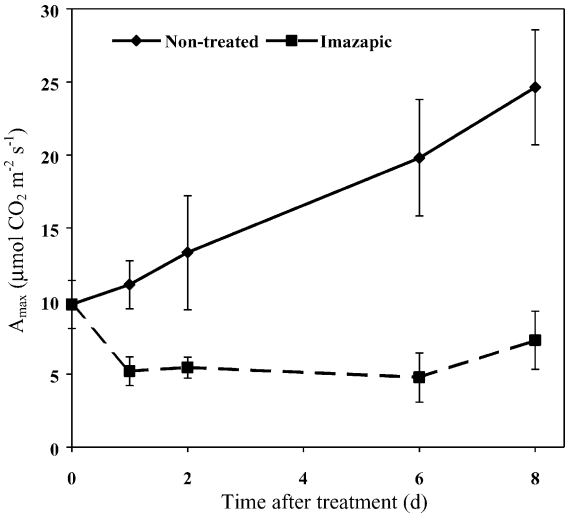

Fig. 2. Photosynthetic rate (A_{max}) response of single non-treated, light-saturated goosegrass leaves and single light-saturated goosegrass leaves treated with imazapic. Error bars indicate standard error of the mean.

non-treated goosegrass may have implications for ACCase inhibition.

Target ACCase is present in rapidly dividing cells and in active chloroplasts [30]. Sethoxydim, a closely related compound to clethodim, rapidly inhibits [^{14}C]acetate incorporation into lipids in corn root tips, but not in the less metabolically active root regions [37]. Visible symptoms of ACCase herbicidal activity are most rapidly and strongly observed in meristematic regions and on an ultrastructural level in the chloroplast [38–40]. Chlorimuron and pyrithiobac did not specifically affect ACCase activity in vitro [13,41], but chlorsulfuron reduced lipid synthesis in isolated soybean leaf cells after 30 min [42]. Imazapic is rapidly absorbed by plants, with greater than 70% of the applied material was absorbed into susceptible and resistant species in the first four hours after treatment [43]. Together, these data suggest that ALS-inhibiting herbicides act very quickly to reduce or halt growth. It has also been demonstrated that 2,4-D and dicamba caused a rapid reduction in the rate of leaf extension in wild and cultivated oats, which affected activity of diclofop-methyl [44]. Similar antagonism of diclofop-methyl efficacy was observed for the same species in response to water stress and nitrogen deficiency [45,46].

Furthermore, metabolism of clethodim in the current study was not affected by the presence of imazapic. By 4 DAT, when goosegrass resumed growth (Fig. 2), and therefore lipid synthesis, clethodim or active species thereof was no longer present in sufficient quantity to inhibit the enzyme (Table 2). Graminicides require actively growing meristematic regions for inhibition of ACCase [18,44]. The data presented herein demonstrate that photosynthetic rate of goosegrass was reduced by treatment with imazapic. Therefore, the requirement for an actively growing plant for herbicidal activity upon ACCase inhibition may be compromised by the reduction of plant growth and photosynthesis caused by ALS inhibition. This growth suppression would reduce plant demand for de novo lipid biosynthesis by ACCase, thus reducing the efficacy of ACCase-inhibiting herbicides.

In addition to inhibiting ACCase, cyclohexanedione herbicides and aryloxyphenoxypropionate herbicides are known to affect plasma membranes of treated plants. Shimabukuro et al. [47] reported a reversal of in vitro aryloxyphenoxypropionate induced plasmalemma perturbation by addition of 2,4-D and hypothesized that this reversal accounted for the observed antagonism between diclofop-methyl and 2,4-D. However, the effects of cyclohexanedione herbicides on plasma membranes are apparently a secondary effect of the perturbation of fatty acid synthesis [48]. The primary cause of antagonism in the majority of the reports have demonstrated that auxin compounds reduced translocation of the graminicides from the treated leaf to the root and shoot [49–53].

Data presented in the current study suggest that the antagonism of clethodim by imazapic may be influenced by imazapic altering the photosynthesis and/or growth rate of goosegrass and therefore the herbicidal consequences of ACCase inhibition. Clethodim was absorbed and translocated similarly to other cyclohexanedione herbicides, and metabolism of clethodim was not affected by the presence of imazapic. Photosynthetic rates of goosegrass, however, were reduced by imazapic treatment. As the plants were not growing, but were metabolizing clethodim, essentially no active herbicide remained in the plant to inhibit any reactivated ACCase. Therefore, imazapic may

prevent the herbicidal activity of the ACCase-inhibiting herbicide clethodim, thus causing the observed antagonism. Further studies are needed to examine whether sensitivity to ACCase-inhibiting herbicides may be influenced by environmental factors that slow or inhibit photosynthesis and growth, as was demonstrated with imazapic treatments.

Acknowledgments

The authors would like to thank Dr. Cavell Brownie, of the Department of Statistics at North Carolina State University, for her expert statistical assistance. Appreciation is also extended to Andy Price for assistance in data collection.

References

[1] J.S. Richburg III, J.W. Wilcut, G.R. Wehtje, Toxicity of AC 263,222 to purple (*Cyperus rotundus*) and yellow nutsedge (*C. esculentus*), Weed Sci. 42 (1994) 398–402.
[2] L.S. Warren Jr., H.D. Coble, Managing purple nutsedge (*Cyperus rotundus*) populations utilizing herbicide strategies and crop rotation sequences, Weed Technol. 13 (1999) 494–503.
[3] J.W. Wilcut, J.S. Richburg III, G.L. Wiley, F.R. Walls Jr., Postemergence AC 263,222 systems for weed control in peanut, Weed Sci. 44 (1996) 615–621.
[4] D.L. Shaner, B.K. Singh, Acetohydroxyacid synthase inhibitors, in: R.M. Roe, J.D. Burton, R.J. Kuhr (Eds.), Herbicide Activity: Toxicology, Biochemistry and Molecular Biology, IOS Press, Burke, VA, 1997, pp. 69–109.
[5] K.M. Jennings, J.W. Wilcut, A.C. York, Grass control with cadre and cadre-graminicide tank mixtures, Proc. APRES 27 (1995) 58.
[6] J.W. Wilcut, J.S. Richburg III, G.L. Wiley, F.R. Walls Jr., Postemergence AC 263,222 systems for weed control in peanut, Weed Sci. 44 (1996) 615–621.
[7] Anonymous, Select 2EC. Crop Protection Reference, 17th ed. C&P Press, New York, 2001, pp. 2606–2614.
[8] D. Ickeringill, Tank mixing—its development—past, present and future, Proc. Aspects Appl. Biol. 41 (1995) 33–39.
[9] I.C. Burke, S.B. Clewis, J.W. Wilcut, Annual grass control with select and cadre, Proc. South. Weed Sci. Soc. 54 (2001) 169.
[10] P.N.P. Chow, Effect of chlorsulfuron on four graminicides for weed control and wheat yield, Weed Res. 28 (1988) 145–150.
[11] A.S. Culpepper, A.C. York, D.L. Jordan, F.T. Corbin, Y.S. Sheldon, Basis for antagonism in mixtures of bromoxynil plus quizalofop-P applied to yellow foxtail (*Setaria glauca*), Weed Technol. 13 (1999) 515–519.
[12] A.S. Culpepper, D.L. Jordan, A.C. York, F.T. Corbin, Y. Sheldon, Influence of adjuvants and bromoxynil on absorption of clethodim, Weed Technol. 13 (1999) 536–541.
[13] K.L. Ferreira, J.D. Burton, H.D. Coble, Physiological basis for antagonism of fluazifop-P by DPX-PE350, Weed Sci. 43 (1995) 184–191.
[14] P.F. Myers, H.D. Coble, Antagonism of graminicide activity on annual grass species by imazethapyr, Weed Technol. 6 (1992) 333–338.
[15] W. Olson, J.D. Nalewaja, Effect of MCPA on ^{14}C-diclofop uptake and translocation, Weed Sci. 30 (1982) 59–63.
[16] J.V. Dean, J.W. Gronwald, C.V. Eberlein, Induction of glutathione S-transferase isozymes in sorghum by herbicide antidotes, Plant Physiol. 92 (1990) 467–473.
[17] R.H. Shimabukuro, W.C. Walsh, R.A. Hoerauf, Metabolism and selectivity of diclofop-methyl in wild oat and wheat, J. Agric. Food Chem. 27 (1979) 615–623.
[18] M.D. Devine, S.O. Duke, C. Fedtke, in: Physiology of Herbicide Action, PTR Prentice-Hall, Englewood Cliffs, NJ, 1993, p. 101.
[19] T.L. Reynolds, Effects of chlorsulfuron, valine, isoleusine on division and tracheary element differentiation in cell suspension cultures of *Solanum carolinense* L, J. Plant Physiol. 125 (1986) 179–184.
[20] T.L. Rost, The comparative cell cycle and metabolic effects of chemical treatments on root tip meristems. III. Chlorsulfuron, J. Plant Growth Regul. 3 (1984) 51–63.
[21] H.D. Bestman, M.D. Devine, W.H. Vanden Born, Herbicide chlorsulfuron decreases assimilate transport out of treated leaves of field pennycress (*Thalspi arvense* L.) seedlings, Plant Physiol. 93 (1990) 1441–1448.
[22] S. Peng, D.R. Krieg, Single leaf and canopy photosynthetic response to plant age in cotton, Agron. J. 83 (1991) 704–708.
[23] N.R. Draper, H. Smith, in: Applied Regression Analysis, 511, Wiley, New York, 1981, pp. 33–42.
[24] [SAS] Statistical Analysis Systems, "SAS/STAT User's Guide, Release 7.00. Statistical Analysis Systems Institute, Cary, NC, 1998, 1028 p.
[25] M.S. McIntosh, Analysis of combined experiments, Agron. J. 75 (1983) 153–155.
[26] G. Wanamarta, D. Penner, Identification of efficacious adjuvants for sethoxydim and bentazon, Weed Technol. 3 (1989) 60–66.
[27] K. Esau, in: Anatomy of Seed Plants, Wiley, New York, 1977, p. 286.
[28] J.D. Burton, J.W. Gronwald, D.A. Somers, J.A. Connelly, B.G. Gengenbach, D.L. Wyse, Inhibition of acetyl-CoA carboxylase by the herbicides sethoxydim and haloxyfop, Biochem. Biophys. Res. Commun. 148 (1987) 1039–1044.
[29] J.R. Campbell, D. Penner, Retention, absorption, translocation, distribution of sethoxydim in monocotyledonous and dicotyledonous plants, Weed Res. 27 (1987) 179–186.
[30] K.A. Croon, M.L. Ketchersid, M.G. Merkle, Effect of bentazon, imazaquin, chlorimuron on the absorption and translocation of the methyl ester of haloxyfop, Weed Sci. 37 (1989) 645–650.

[31] M. Focke, H.K. Lichtenthaler, Inhibiton of acetyl-CoA carboxylase of barley chloroplasts by cycloxydim and sethoxydim, A. Naturforsch 42c (1987) 1361–1363.
[32] A.R. Rendina, J.M. Felts, Cylcohexanedione herbicides are selective and potent inhibitors of acetyl-CoA carboxylase from grasses, Plant Physiol. 86 (1988) 983–986.
[33] Y.K. Hashimoto, I. Iwataki, Y. Soeda, Fate of alloxydim-sodium on or in soybean plants, Pest. Sci. 4 (1979) 299–304.
[34] V. Soeda, K. Ishihara, I. Iwataki, H. Kamimura, Fate of a herbicide ^{14}C-alloxydim-sodium in sugar beet, Pest. Sci. 4 (1979) 121–128.
[35] F.M. Ashton, A.S. Crafts, in: Mode of Action of Herbicides, second ed., Wiley, New York, NY, 1981, pp. 221–222.
[36] J.R. Campbell, D. Penner, Sethoxydim metabolism in monocotyledonous and dicotyledonous plants, Weed Sci. 33 (1985) 771–773.
[37] H. Hosaka, M. Takagi, Biochemical effects of sethoxydim in excised root tips of corn (*Zea mays*), Weed Sci. 35 (1987) 612–618.
[38] A.G. Brezeanu, D.G. Davis, R.H. Shimabukuro, Ultrastructural effects and translocation of methyl 2-[4-(2,4-dichloro-phenoxy)phenoxy]proponoate in wheat (*Triticum aestivum*) and wild oat (*Avena fatua*), Can. J. Bot. 53 (1976) 2038–2048.
[39] J.P.N.R. Chandrasena, G.R. Sagar, Effect of fluazifop-butyl on the chlorophyll content, fluorescence and chloroplast ultrastructure of *Elymus repens* (L.) Gould. leaves, Weed Res. 27 (1987) 103–112.
[40] R. Jain, W.H. Vander Born, Morphological and histological effects of three grass selective herbicides on developing wild oat (*Avena fatua*) stems, Weed Sci. 37 (1989) 575–584.
[41] L.A. Bjelk, T.J. Monaco, Effect of chlorimuron and quizalofop on fatty acid biosynthesis, Weed Sci. 40 (1992) 1–6.
[42] K.K. Hatzios, C.M. Howe, Influence of the herbicides hexazinone and chlorsulfuron on the metabolism of isolated soybean leaf cells, Pest. Biochem. Physiol. 17 (1982) 207–214.
[43] L.J. Newsom, D.R. Shaw, T.F. Hubbard Jr., Absorption, translocation, and metabolism of AC 263,222 in peanut (*Arachis hypogaea*), soybean (*Glycine max*), and selected weeds, Weed Sci. 41 (1993) 523–527.
[44] M. Andrews, Diclofop-methyl antagonism by broadleaf weed herbicides: the importance of leaf expansion rates, Weed Res. 30 (1990) 331–340.
[45] M. Andrews, R.L. Dickson, M.H. Forman, F. Dastgheib, R.J. Field, The affects of different external nitrogen concentrations on growth of *Avena sativa* cv. Amuri treated with diclofop-methyl, Ann. Appl. Biol. 114 (1989) 339–348.
[46] F. Dastgheib, F.M. Andrews, R.J. Field, M.H. Foreman, Effect of manitol induced water stress on the tolerance of cultivated oat (*Avena sativa* L.) to diclofop-methyl, Weed Res. 30 (1990) 171–179.
[47] R.H. Shimabukuro, W.C. Walsh, J.P. Wright, Effect of diclofop-methyl and 2,4-D on transmembrane proton gradient: a mechanism for their antagonistic interaction, Physiol. Plant. 77 (1989) 107–114.
[48] J.M. Di Tomaso, Evidence against a direct membrane effect in the mechanism of action of graminicides, Weed Sci. 42 (1994) 302–309.
[49] G.R. Gillespie, J.D. Nalewaja, Influence of 2,4-D and MCPA formulations and oil on diclofop phytotoxicity, Weed Sci. 37 (1989) 380–384.
[50] B. Kafiz, J.P. Caussanel, R. Scalla, P. Gailardon, Interaction between diclofop-methyl and 2,4-D in wild oat (*Avena fatua* L.) and cultivated oat (*Avena sativa* L.), and fate of diclofop-methyl in cultivated oat, Weed Res. 29 (1989) 299–305.
[51] T.C. Mueller, M. Barrett, W.W. Witt, A basis for the antogonistic effect of 2,4-D on haloxyfop-methyl toxicity to johnsongrass (*Sorghum halepense*), Weed Sci. 38 (1990) 103–107.
[52] W. Olson, J.D. Nalewaja, Effect of MCPA on ^{14}C-diclofop uptake and translocation, Weed Sci. 30 (1982) 59–63.
[53] B.G. Todd, E.H. Stobbe, The basis of the antagonistic effect of 2,4-D on diclofop-methyl toxicity to wild oat (*Avena fatua*), Weed Sci. 28 (1980) 371–377.